

CLAIMS

- Sub a2
1. A purified decoy probe comprising,
a first nucleotide base recognition sequence region, wherein said first region binds
5 to an RNA polymerase, and
an optionally present second nucleotide base recognition sequence region,
provided that if said first region is nucleic acid, then said second region is either
directly joined to the 5' end of said first region or is joined to the 3' end or 5' end of said
first region by a non-nucleotide linker,
10 wherein said optionally present second region is present if said first region can be
used to produce a functional double-stranded promoter sequence using a complementary
oligonucleotide,
further provided that if said first region is nucleic acid which can be used to
produce said functional double-stranded promoter sequence using said complementary
15 oligonucleotide, then said decoy probe does not have a nucleic acid sequence greater than
about 10 nucleotides in length joined directly to the 3' end of said first region.
2. The decoy probe of claim 1, wherein said first region is nucleic acid, said
second region is directly joined to the 5' end of said first region, and said decoy probe does
20 not have a nucleotide base sequence greater than 10 nucleotides in length joined directly to
its 3' end.
3. The probe of claims 1, wherein said probe consists of 15 to 100 optionally
modified nucleosides and one or more blocking groups located at the 3' terminus of said
25 probe, wherein each of said optionally modified nucleosides independently has,
a purine or pyrimidine moiety independently selected
from the group consisting of inosine, uracil, adenine, guanine, thymine and
cytosine; and
a sugar moiety independently selected from the group
30 consisting of deoxyribose, 2'-methoxy ribose, and ribose; and

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each of said optionally modified nucleosides is joined together by an internucleoside linkage independently selected from the group consisting of phosphodiester, phosphorothioate, and methylphosphonate.

- 5 4. The probe of claim 3, wherein
 at least 80% of said optionally modified nucleosides have a purine or pyrimidine moiety independently selected from the group consisting of adenine, guanine, thymine and cytosine, and a deoxyribose sugar moiety; and
 at least 80% of said internucleoside linkages joining said optionally modified
10 nucleosides are phosphodiester.

 5. The probe of claim 4, wherein said probe consists of 15 to 100 independently selected deoxyribonucleotides and one or more blocking groups located at the 3' terminus of said probe.

- 15 6. The probe of claim 3, wherein said one or more blocking groups are selected from the group consisting of phosphorothioate, alkane-diol residue, cordycepin, and an alkyl group.

Sub A 3 7. The probe of claim 6, wherein said probe consists of 35 to 70 independently selected nucleotides, said one or more blocking groups, and said second
20 region comprises at least 10 nucleotides.

 8. The probe of claim 7, wherein said RNA polymerase is T7 RNA polymerase.

 9. The probe of claim 7, wherein said RNA polymerase is T3 RNA polymerase.

- 25 10. The probe of claim 7, wherein said RNA polymerase is SP6 RNA polymerase.

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11. A purified decoy probe comprising,
a first nucleotide base recognition sequence region, wherein said first region has at
least 35% sequence similarity to an RNA polymerase promoter sequence, and
an optionally present second nucleotide base recognition sequence region,
5 provided that if said first region is nucleic acid, then said second region is either
directly joined to the 5' end of said first region or is joined to the 3' end or 5' end of said
first region by a non-nucleotide linker,
wherein said optionally present second region is present if said first region can be
used to produce a functional double-stranded promoter sequence using a complementary
10 oligonucleotide,
further provided that if said first region is nucleic acid which can be used to
produce said functional double-stranded promoter sequence using said complementary
oligonucleotide, then said decoy probe does not have a nucleic acid sequence greater than
about 10 nucleotides in length joined directly to the 3' end of said first region.

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12. The decoy probe of claim 11, wherein said first region is nucleic acid, said
second region is directly joined to the 5' end of said first region, and said decoy probe does
not have a nucleotide base sequence greater than 10 nucleotides in length joined directly to
its 3' end.

13. The probe of claim 11, wherein said probe consists of 15 to 100 optionally
20 modified nucleosides and one or more blocking groups located at the 3' terminus of said
probe, wherein each of said optionally modified nucleosides independently has,
a purine or pyrimidine moiety independently selected
from the group consisting of inosine, uracil, adenine, guanine, thymine and
cytosine; and
25 a sugar moiety independently selected from the group
consisting of deoxyribose, 2'-methoxy ribose, and ribose; and
each of said optionally modified nucleosides is joined together by an internucleoside
linkage independently selected from the group consisting of phosphodiester,
phosphorothioate, and methylphosphonate.

14. The probe of claim 13, wherein
at least 80% of said optionally modified nucleosides has a purine or pyrimidine
moiety independently selected from the group consisting of adenine, guanine, thymine and
cytosine, and a deoxyribose sugar moiety; and

5 at least 80% of said internucleoside linkages joining said optionally modified
nucleosides are phosphodiester.

Sub 696 15. The probe of claim 14, wherein said probe consists of 35 to 70
independently selected nucleotides, one or more blocking groups, and said second region
10 comprises at least 10 nucleotides.

16. The probe of claim 13, wherein said one or more blocking groups are
selected from the group consisting of phosphorothioate, alkane-diol residue, cordycepin,
and an alkyl group.
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17. The probe of claim 16, wherein said first region has a nucleotide base
sequence similarity of at least 75% with at least one of SEQ ID Nos. 1, 2, 3, 4, 5 and 6.

18. The probe of claim 17, wherein said first region has a sequence similarity
20 of 75% to 95% with SEQ ID NO: 3.

19. A reagent mixture for use in an amplification reaction comprising a nucleic
acid polymerase and a reversible inhibitor of said polymerase, wherein said reagent
mixture does not contain a nucleic acid substantially complementary to said inhibitor.
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20. The reagent mixture of claim 19, wherein said reagent mixture does not
contain an oligonucleotide having a 3' OH available for a primer extension reaction, and
said inhibitor is not a substrate in a primer extension reaction.

21. The reagent mixture of claim 19, wherein said nucleic acid polymerase is
an RNA polymerase.
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22. The reagent mixture of claim 21, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence having at least 35% sequence similarity to an RNA polymerase promoter sequence.

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23. A method for amplifying a target nucleic acid sequence under amplification conditions comprising the steps of:

- a) producing a mixture comprising an amplification enzyme and a reversible inhibitor of said enzyme, wherein said reversible inhibitor does not hybridize to a target nucleic acid comprising said target nucleic acid sequence under said amplification conditions, and wherein said mixture does not contain said target nucleic acid; and
- 10 b) providing said mixture to said target nucleic acid, and
- c) amplifying said target nucleic acid sequence under said amplification conditions.

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24. The method of claim 23, wherein said method is a transcription-associated amplification, prior to said step (b) no amplification oligonucleotides used in said transcription-associated amplification are present, and said amplification enzyme is an RNA polymerase.

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25. The method of claims 24, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence region which binds to said RNA polymerase.

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26. The method of claims 24, wherein said inhibitor is a decoy probe comprising a nucleotide base recognition sequence having at least 35% sequence similarity to a promoter sequence recognized by said RNA polymerase sequence.

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27. A transcription-associated amplification procedure comprising the step of amplifying a target nucleic acid sequence to produce multiple copies of RNA transcripts by combining together under transcription-associated amplification conditions a mixture

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comprising a target nucleic acid comprising said target nucleic acid sequence, a promoter-template complementary probe, a DNA polymerase, an RNA polymerase, ribonucleoside triphosphates, deoxyribonucleoside triphosphates, and means for reversibly inhibiting said RNA polymerase, wherein said means for reversibly inhibiting said RNA polymerase does
5 not hybridize to a target nucleic acid comprising said target nucleic acid sequence under said amplification conditions to form a stable inhibitor:target complex.

28. The method of claim 27, wherein said DNA polymerase is a reverse transcriptase and said means for reversibly inhibiting said RNA polymerase is not a
10 substrate in a primer extension reaction.

29. The method of claim 28, wherein said RNA polymerase, said reverse transcriptase, and said means for reversibly inhibiting said RNA polymerase are first combined together in the absence of said promoter-template complementary probe.
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30. An improved method of amplifying a nucleic acid, wherein the improvement comprises the step of providing a nucleic acid polymerase with means for reversibly inhibiting said polymerase prior to providing said polymerase to said nucleic acid.
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31. The method of claim 30, wherein said means for reversibly inhibiting said polymerase is not a substrate in a primer extension reaction.

32. The method of claim 31, wherein said amplifying is a transcription-associated amplification.
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33. The method of claim 32, wherein said amplifying is a strand displacement amplification.

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